MRID No. 407571-01

DATA EVALUATION RECORD

1. **CHEMICAL:** DEF. Shaughnessey Number: Not available.

2. **TEST MATERIAL:** DEF; Tribufos Technical (S,S,S-tributyl phosphorotrithioate), a light amber liquid. 98.7% purity.

3. **STUDY TYPE:** Avian Reproduction Study.
Species Tested: Bobwhite quail (Colinus virginianus).

4. **CITATION:** Beavers, J.B., G. Marselas, and M. Jaber. 1988. A One-generation Reproduction Study with the Bobwhite (Colinus virginianus). Laboratory Project No. 149-127. Prepared by Wildlife International, Ltd., Easton, Maryland. Submitted by Mobay Corporation, Stilwell, Kansas. MRID No. 407571-01.

5. **REVIEWED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 5-19-89

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: James R Newman
Date: 5/24/89

Reviewed by: Art Reybal EEB Wildlife Biologist

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature:
Date: 12-18-89

7. **CONCLUSIONS:** Nominal dietary concentrations of DEF at 410 ppm resulted in reduced egg production and survival of hatchlings. Necropsies of birds in the 410 ppm group at study termination showed an increased number of females with a regressed or regressing ovary, and slight to moderate distension of the gastrointestinal tract in all birds of this group. Egg shell thickness was decreased in the 280 ppm group. The NOEC was 150 ppm. This study is scientifically sound and meets the requirements for an avian reproductive test.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds employed in this study were unmated 20 week-old Bobwhite quail received from Sand Prairie Quail Farm, Maquoketa, Iowa. All birds were acclimated to the facilities for 4 weeks prior to initiation of the test. Birds that did not appear healthy at test initiation were discarded.

B. **Dose/Diet Preparation/Food Consumption:** Test diets were prepared by mixing DEF into a pre-mix which was used for preparation of the final diet. Control diet and three test concentrations (150, 280 and 410 ppm) were prepared weekly and presented to birds on Thursday of each week. The control diet contained an amount of the carrier (corn oil) and solvent (acetone) equal to that in the treated diets. Adults were fed a game bird ration formulated for breeding birds. All offspring received a game bird ration formulated for young growing birds. The test substance was not mixed into the diet of the offspring. Water and feed were supplied ad libitum during acclimation and during the test.

Samples of the control diet and each of the test diets were taken weekly after mixing and frozen immediately after collection. Diet samples were analyzed for weeks 1, 2, 5, 10, 15, and 20. Food consumption in each pen was determined weekly throughout the study.

C. **Design:** The birds were randomly distributed into four groups as follows:

Nominal Concentration	Number Of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	16	1	1
150 ppm	16	1	1
280 ppm	16	1	1
410 ppm	16	1	1

"Treatment levels were based upon known toxicity data and consultation with the client." Adult birds were identified by individual leg bands. The primary phases

of the study and their approximate durations were as follows:

1. Acclimation - 4 weeks.
2. Pre-photostimulation - 8 weeks.
3. Pre-egg laying (with photostimulation) - 4 weeks.
4. Egg laying - 9 weeks.
5. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 6 weeks.

D. **Pen Facilities**: Adult birds were housed indoors in 30 cm x 51 cm galvanized wire pens. The pens had sloping floors which resulted in a ceiling height ranging from 21 to 26 cm. The average temperature in the adult study room was 22.3°C ± 3.4°C (SD) with an average relative humidity of 53%.

During acclimation and upon initiation of the study, the birds were maintained under a photoperiod of eight hours of light per day. The photoperiod was increased to 17 hours of light per day during week 8, and was maintained at that length until terminal sacrifice. Birds received approximately 12 footcandles of illumination throughout the study.

E. **Adult Observations/Gross Pathology**: All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. All birds that died during the study were necropsied. At study termination, all surviving birds were sacrificed and necropsied. Adult birds were weighed at test initiation, at the end of weeks 2, 4, 6, 8, and at study termination.

F. **Eggs/Eggshell Thickness**: Eggs were collected daily, marked according to pen of origin, and fumigated to prevent pathogen contamination. The eggs were then stored at 11.2°C ± 1.2°C and 75% relative humidity until incubated. Eggs were removed from the storage room weekly and candled. Cracked or abnormal eggs were discarded. All eggs that were not cracked, abnormal or used for egg shell thickness measurements were placed in an incubator at 37.4°C and 56% relative humidity. Eggs were candled again on day 11 of incubation to determine embryo viability and on day 21 to determine embryo survival. All eggs were turned automatically while in the incubator and placed in hatching trays on incubation day 21. Temperature in the hatcher was 37.1°C with a relative humidity of 70%.

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during the odd numbered weeks, and from each of the even numbered pens during the even numbered weeks. These eggs were used for egg shell thickness measurements. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.005 mm) five points around the waist of the egg using a micrometer.

G. **Hatchlings**: All hatchlings and unhatched eggs were removed from the hatcher on day 25 or 26 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were leg banded for identification by pen of origin and then placed in galvanized wire mesh brooding pens until 14 days of age. Each brooding pen measured 72 cm x 90 cm x 23 cm high. Brooder temperatures were maintained at 38°C. The photoperiod was maintained at 16 hours of light per day. Hatchlings were fed untreated diet. At 14 days of age the average body weight by parental pen of all survivors was determined.

H. **Statistics**: Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following arcsine transformation. The pens in which mortality occurred were not used in statistical comparisons of the data. Each of the following parameters was analyzed statistically:

Adult Body Weight
Adult Feed Consumption
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-Week Embryos of Viable Embryos
Hatchlings of 3-Week Embryos
Hatchlings of Eggs Set
Offspring's Body Weight
Hatchlings of Maximum Set
14-Day Old Survivors of Maximum Set
14-Day Old Survivors of Eggs Set
14-Day Old Survivors of of Hatchlings
Egg Shell Thickness

12. REPORTED RESULTS

A. **Diet Analysis**: The mean measured concentration of DEF in the diets was 99%, 94%, and 96% of nominal

concentration for the 150, 280, and 410 ppm groups, respectively. (Reviewer's note: The authors, in all subsequent references to the treatment groups, refer to the groups by the mean measured concentrations, i.e., 148 ppm, 262 ppm, and 392 ppm).

B. **Mortality and Behavioral Reactions**: There were no mortalities in the control group or the 280 ppm group.

Three mortalities occurred in the 150 ppm group and three in the 410 ppm group. All mortalities except one appeared to be unrelated to treatment. One mortality in the 410 ppm group may have been treatment related.

The first mortality in the 150 ppm group was a female found dead during week 7. Necropsy revealed a cut on the right foot, and bruises on the head and neck. The second death, a female, was first noted to have head lesions during week 12. Necropsy revealed a bird light in body weight (166 g) with extensive head lesions. The third mortality in the 150 ppm group was a female that died during week 16. Extensive egg yolk peritonitis was discovered during necropsy.

The first mortality in the 410 ppm group was a female that sustained a broken leg during body weight measurements at the end of week 2 and was euthanized. The second mortality, a female, died at the end of week 10. In the week prior to her death, the bird had exhibited a ruffled appearance, wing droop, depression (reduced activity), and reduced reaction to external stimuli. Necropsy revealed emaciation (body weight of 110 g) and feather loss on the head. Internally, the ceca were distended with fluid and gas. The test chemical appeared to have been at least contributory to that mortality. The third mortality in this group was a male found during week 11. The bird had been noted earlier in the week with head lesions and displaying lethargy and a ruffled appearance. Necropsy revealed extensive head lesions with bone exposed on the cranium.

Necropsies of pen mates of all six mortalities were unremarkable.

No overt signs of toxicity were observed in any group. Respiratory signs such as coughing were observed in 3-8 birds from each group. These signs were first observed during week 4 and had abated by week 8. Based on tissue cultures of extra birds from this lot, the Animal Health Laboratory of the Maryland Department of Agriculture

made a diagnosis of quail bronchitis. "Since this disease is generally self limiting in adult birds, no attempts were made to treat the affected birds, all of which recovered uneventfully".

"Other clinical signs not attributed to treatment, such as intermittent lethargy or depression, reduced reaction to external stimuli (sound and movement), ruffled appearance, wing droop or head curl, were observed in a few birds in all treatment groups during the course of the study. Except for the mortalities and clinical signs previously noted, and aside from lesions or observations normally associated with pen wear and/or interaction among pen mates, all other birds at all concentrations appeared normal throughout the study."

Necropsy of all surviving adults was conducted at study termination. All lesions observed in the 150 ppm and 280 ppm groups were considered to be incidental and not related to treatment. Necropsies of birds in the 410 ppm group showed an apparent increase in the number of females with a regressed or regressing ovary, and slight to moderate distension of the gastrointestinal tract in all birds (Appendix IV, attached).

C. **Adult Body Weight and Food Consumption**: No significant differences in body weights between the control and any treatment group were noted throughout the investigation.

Food consumption varied between pens due to excessive wastage by some birds. When compared to the control group, there were slight but significant ($p<0.01$) increases in food consumption in the 150 ppm group during weeks 16-20. These differences were considered incidental to treatment. At 410 ppm, there were slight but significant ($p<0.05$) increases in food consumption during weeks 5, 6, 14, and 18 and significant ($p<0.01$) increases during weeks 7, 11, 13, 15, 16, 17, 19, and 20. (Table 2, attached). "These increases, particularly those that occurred during the early portion of the study, may have been treatment related."

D. **Reproduction**: When compared to controls, the 150 ppm group showed a slight, but significant ($p<0.05$) decrease in the number of cracked eggs as a percentage of eggs laid. At 280 ppm, there was a slight reduction in the number of eggs laid, but the difference was not statistically significant. There were no other significant or apparent effects upon any reproductive

parameter at the 150 or 280 ppm concentrations (Tables 3 & 3A, attached).

"At 392 ppm, there was a marked effect upon reproductive performance with statistically significant ($p<0.01$) effects upon the numbers of eggs laid, the number of 14-day old survivors as a percentage of the number of hatchlings, the number of hatchlings as a percentage of the maximum number of eggs set, and the number of 14-day old survivors as a percentage of the number of eggs set and the maximum number of eggs set. While not statistically significant, there was also a reduction in the number of viable embryos as a percentage of eggs set." (Tables 3 & 3A, attached).

E. **Egg Shell Thickness**: When compared to the control group, there were no significant differences in egg shell thickness at 150 or 410 ppm. At 280 ppm, when compared to the control group, there was a slight, but significant ($p<0.05$) decrease in egg shell thickness (Table 4, attached).

F. **Offspring Body Weight and Survival**: Fifteen offspring from the 150 ppm group, 18 from the 280 ppm group, and two from the 410 ppm group were found dead after the failure of one brooder on January 26, 1988. These mortalities were incidental to treatment and thus were not included when computing the percentage of 14-day survivors.

There were no significant differences in body weights of hatchlings at any test concentration. There were no significant differences in body weights of 14-day old survivors at the 150 and 280 ppm concentrations. There was a slight but significant ($p<0.01$) decrease in body weights of 14-day old survivors at 410 ppm (Tables 5 & 5A, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

Dietary concentrations of DEF at 150 ppm did not result in treatment related mortality, overt signs of toxicity or effects upon reproductive performance during the 20 week exposure period. At 280 ppm, there may have been a slight reduction in egg production, but this reduction was not statistically significant. There was a significant ($p<0.05$) decrease in egg shell thickness at 280 ppm. At 410 ppm, there were pronounced effects upon reproductive parameters and there may have been one treatment related mortality. The no-observed-effect concentration was 150 ppm.

The study was conducted in conformance with Good Laboratory Practice regulations. The data were inspected and the final report signed by the Quality Assurance Officer of Wildlife International, Ltd.

14. **Reviewer's Discussion and Interpretation of the Study:**

A. **Test Procedures:** The test procedures were in accordance with the SEP and Subdivision E guidelines except for the following deviations:

Adult birds were exposed to 12 foot-candles of illumination; 6 foot-candles is recommended.

Eggs were stored at a temperature of 11°C and a relative humidity of approximately 75%; 16°C and 65% are recommended.

Eggs were candled on day 21 of incubation to determine embryo survival; day 18 is recommended by the SEP.

A withdrawal study period was not added to the test phase.

Behavioral observations of offspring were not reported.

B. **Statistical Analysis:** Statistical procedures differed from recommended methods. Specifically, there is no basis for transforming the number of eggs laid and the number of hatchlings to percentile values of the maximum number of eggs laid or set in any test group, which were then used in statistical procedures.

Analyses of reproductive parameters were verified (attached) and generally matched those reported by the authors. The values for 14 day survivors/eggs set, however, did not significantly differ between groups, contrary to the authors' results. Additionally, the 410 ppm values differed from the control ($p<0.05$) for the following parameters which were not analyzed by the authors: eggs set, viable embryos, live 3-week embryos, number of hatchlings, and 14-day old survivors.

C. **Discussion/Results:** Egg production, survival of hatchlings, and body weight of 14-day survivors were decreased in the 410 ppm group. Necropsies of survivors showed an increase in the number of females with a regressed or regressing ovary, and slight to moderate distension of the gastrointestinal tract in all birds of

this group. Food consumption may have been affected and one mortality may have been treatment related at 410 ppm.

There was a significant ($p<0.05$) decrease in egg shell thickness at 280 ppm and a slight, but non-significant reduction in the number of eggs laid.

The increased food consumption noted in the 150 ppm group during weeks 16-20 probably was not treatment related. The NOEC of 150 ppm reported by the author is thus accepted.

This study is scientifically sound and meets the requirements for an avian reproductive test.

D. **Adequacy of the Study:**

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. **COMPLETION OF ONE-LINER:** Yes, May 19, 1989.

APPENDIX IV

GROSS PATHOLOGICAL OBSERVATIONS

DEF - PROJECT NUMBER 149-127

BIRDS SACRIFICED AT TERMINATION OF THE STUDY

	MALES				FEMALES			
	PPM				PPM			
	CONTROL	150	280	410	Control	150	280	410
Slight Head Lesions	1/16	0/13	0/16	0/13	0/16	0/13	0/16	0/13
Head Lesions	0/16	0/13	0/16	0/13	0/16	2/13	0/16	0/13
Extensive Head Lesions	0/16	0/13	0/16	0/13	0/16	0/13	0/16	0/13
Slight Foot Lesions	0/16	0/13	0/16	0/13	0/16	0/13	0/16	0/13
Foot Lesions	2/16	5/13	0/16	2/13	4/16	4/13	5/16	0/13
Extensive Foot Lesions	0/16	2/13	0/16	0/13	1/16	1/13	0/16	0/13
Slight Feather Loss	2/16	0/13	0/16	0/13	6/16	3/13	3/16	5/13
Feather Loss	1/16	0/13	0/16	1/13	0/16	1/13	1/16	0/13
Extensive Feather Loss	0/16	0/13	0/16	0/13	0/16	1/13	2/16	1/13
Slight Egg Yolk Peritonitis	0/16	0/13	0/16	0/13	2/16	0/13	2/16	0/13
Egg Yolk Peritonitis	0/16	0/13	0/16	0/13	1/16	1/13	2/16	1/13
Regressing Ovary	0/16	0/13	0/16	0/13	0/16	0/13	0/16	3/13
Regressed Ovary	0/16	0/13	0/16	0/13	0/16	1/13	1/16	3/13

(Continued)

APPENDIX IV

GROSS PATHOLOGICAL OBSERVATIONS

DEF - PROJECT NUMBER 149-127

BIRDS SACRIFICED AT TERMINATION OF THE STUDY

PAGE 2

	MALES				FEMALES			
	PPM				PPM			
	CONTROL	150	280	410	Control	150	280	410
Regressing Testes	0/16	1/13	0/16	0/13	0/16	0/13	0/16	0/13
Regressed Testes	0/16	1/13	0/16	0/13	0/16	0/13	0/16	0/13
Slight to Moderate Distention of Intestinal Tract	0/16	0/13	0/16	13/13	0/16	0/13	0/16	13/13
Severe Distention of Intestinal Tract	0/16	0/13	0/16	0/13	0/16	0/13	0/16	1/13
Not Remarkable	12/16	6/13	16/16	0/13	3/16	5/13	5/16	0/13

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TABLE 2

MEAN FEED CONSUMPTION DATA (Grams/Bird/Day)

BOBWHITE

DEF - PROJECT NUMBER 149-127

WEEK	0 PPM	150 PPM	280 PPM	410 PPM
1	12	13	12	12
2	14	15	14	15
3	16	15	15	17
4	15	17	17	18
5	13	13	15	17 *
6	18	16	16	21 *
7	15	15	15	19 **
8	17	17	17	19
9	18	18	17	19
10	19	19	18	20
11	21	22	20	25 **
12	23	24	21	26
13	21	22	21	26 **
14	22	24	23	27 *
15	25	28	24	30 **
16	25	30 **	24	30 **
17	26	31 **	26	31 **
18	28	33 **	27	31 *
19	28	34 **	28	34 **
20	27	31 **	27	31 **

* Difference from the control statistically significant at $p < .05$.
**Difference from the control statistically significant at $p < .01$.

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TABLE 3

REPRODUCTIVE DATA - BOBWHITE

DEF - PROJECT NUMBER 149-127

	DEF			
	0 PPM	150 PPM	280 PPM	410 PPM
Eggs Laid	633	518	518	181
Eggs Cracked	6	0	1	4
Eggs Set	563	466	457	137
Viable Embryos	480	381	372	101
Live 3-Week Embryos	476	378	370	99
Hatchlings	427	336	335	84
14-Day Old Survivors	377	289	277	51
Eggs Laid/Hen	40	40	32	14
Eggs Laid/Hen/Day @	0.61	0.61	0.50	0.21
14-Day Old Survivors/Hen	24	22	17	4

@ - Based on 65 days.

TABLE 3A

REPRODUCTIVE DATA - (%) - BOBWHITE

DEF - PROJECT NUMBER 149-127

	DEF			
	0 PPM	150 PPM	280 PPM	410 PPM
Eggs Laid	633	518	518	181
Eggs Laid/Max. Laid (%)	63	63	51	22 **
Eggs Cracked/Eggs Laid (%)	1	0 *	0	2
Viable Embryos/Set (%)	84	83	80	69
Live 3-Week Embryos/Viable (%)	99	99	100	99
Hatchlings/3-Week (%)	90	88	88	85
14-Day Old Survivors/Hatch (%)	88	91	86	56 **
Hatchlings/Set (%)	75	73	72	59
14-Day Old Survivors/Set (%)	66	66	60	29 **
Hatchlings/Max. Set (%)	47	45	37	11 **
14-Day Old Survivors/Max. Set (%)	41	39	30	7 **

* Difference from the control statistically significant at $p < .05$.
**Difference from the control statistically significant at $p < .01$.

PROJECT NO.: 149-127

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TABLE 4

EGG SHELL THICKNESS DATA - (mm)

BOBWHITE

DEF - PROJECT NUMBER 149-127

	DEF			
	0 PPM	150 PPM	280 PPM	410 PPM
No. of Eggs Measured	62	50	59	31
Mean Egg Shell Thickness (mm)	0.227	0.219	0.212 *	0.220
± standard deviation	± 0.015	± 0.015	± 0.015	± 0.025

*Difference from the control statistically significant at $p < .05$.

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TABLE 5
BODY WEIGHT DATA (g) - HATCHLINGS
BOBWHITE
DEF - PROJECT NUMBER 149-127

	DEF			
	0 PPM	150 PPM	280 PPM	410 PPM
No. of Chicks Weighed	427	335	331	84
Mean Body Weight (g)	6 ± 1	6 ± 1	5 ± 0	5 ± 1

The above differences from the control are not statistically significant.

TABLE 5A
BODY WEIGHT DATA (g) - 14-DAY SURVIVORS
BOBWHITE
DEF - PROJECT NUMBER 149-127

	DEF			
	0 PPM	150 PPM	280 PPM	410 PPM
No. of Chicks Weighed	377	289	277	51
Mean Body Weight (g)	26 ± 4	24 ± 3	23 ± 4	20 ± 4 **

** = Difference from the control statistically significant at $p < .01$.

Shaughnessey No. Not available Chemical Name DEF Chemical Class _____ Page 1 of 1

Study/Species/Lab/ Succession	Chemical % Active	Results				Reviewer/ Date	Valid Stat
Avian Reproduction,		Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%Cha Inh.	
Species: Bobwhite		Control	0	NONE	0	N/A	M.L. WHITTEN 5-19-89 CORE
(Colinus virginianus)	98.7%	Treatment I	150	NONE	9%		
Lab: Wildlife International, Ltd.		Treatment II	280	EGG SHELL THICKNESS	0		
~~Acc~~ MRID # 407571-01		Treatment III	410	EGG PRODUCTION, # 14 DAY SURVIVORS, WEIGHT OF 14 DAY SURVIVORS, DISTENDED GI TRACTS, REGRESSED OVARIES	9%		
		Study Duration: 20 WEEKS					
		Comments:					

Field Study(Simulated/Actual) Species:	Group	Rate(ai/a)	Treatment Interval	Total # Treatments	Mor.(%)		
	Control						
_____	Treatment I						
Lab:	Treatment II						
Acc.	Treatment III						
	Crop/Site:		Study Duration:				
	Comments:						

Chronic fish, Species _____ Lab: _____ Acc.

Concentrations Tested (pp__)= _____

MATC = >____ <____ pp__. Effected Parameter = _____

Contr. Mort.(%)= _____ Sol. Contr. Mort.(%)= _____ _____ _____

Comments:

Chronic invertebrate Species _____ Lab Acc.

Concentrations Tested (pp__)= _____

MATC =>____ <____ pp__. Effected Parameter(s) _____

Contr. Mort.(%)= _____ Sol. Contr. Mort.(%)= _____ _____ _____

Comments: